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ABSTRACTS FROM THE ORIGINAL PAPERS.

The Effect of Acid Hydrolysis upon ~~S~~Cystine, and the Determination of Cystine in Protein.

(*The third paper on Sulphur Containing Amino-acids.*)

By Yuzuru Okuda and Junjirō Motomura.
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1. The effect of acid hydrolysis upon cystine :- It seems that the first important question to be solved for the determination of cystine in proteins is whether cystine is decomposed or not during acid hydrolysis. Van Slyke (J. Biol. Chem., **10**, 38, 1911) found that when cystine was boiled with 20% HCl for 24 hours, only 50% was precipitated by phosphotungstic acid, and he stated that it appears possible that the cystine is partially destroyed during the hydrolysis. Plimmer (Biochem. J., **7**, 311, 1913) also found that cystine is decomposed by boiling with conc. HCl for 5-8 hours. From these descriptions we see that cystine is pretty unstable when boiled with mineral acid. If it is true, it is difficult to determine cystine in proteins after acid hydrolysis. But on the contrary Gortner and Holm (J.Am. Chem. Soc., **42**, 821, 1920) found that cystine was not readily deaminized. Namely they boiled some amino acid mixture containing cystine with 20% HCl for 24 hours, and showed that if all of the ammonia nitrogen was calculated as being derived from the cystine, only less than 3% of cystine has been changed. Lately Hoffman and Gortner (J. Am. Chem. Soc., **44**, 341, 1922) also determined cystine, after prolonged boiling with HCl, by Okuda's bromine method (J. Coll. Agr. Imp. Univ. Tokyo, **7**, 69, 1919), and came to the conclusion that there is no appreciable decomposition

of cystine during ordinary acid hydrolysis of proteins, but the amount of cystine precipitable as phoshotungstate decreases, as a part of the cystine is transformed to its isomer, whose phosphotungstate is readily soluble.

In this present paper the authors have ascertained the results obtained by Gortner and his co-workers. In the first experiments, pure cystine was boiled with 20 % HCl, or 25% H_2SO_4 respectively, for 30 hours and cystine was determined in the solution by the bromine method. In the second group of experiments some human hair was hydrolysed by boiling with fifteen times its weight of HCl in different concentrations as 20, 25 and 38%. After a certain time from 15 to 30 hours, of boiling under a reflex condenser, 10 c.c. of the solution was withdrawn and cystine was determined by Okuda's iodine method (J. Chem. Soc. Japan, **45**, 18, 1924), and Folin and Loony's colorimetric method (J. Biol. Chem., **51** 421, 1922). In every experiment performed, no appreciable decomposition of cystine was observed during the time of acid hydrolysis.

2. The determination of cystine in proteins :- The bromine method is a very rapid and accurate method for the determination of cystine, but it can not be used directly in the presence of tyrosine and tryptophan. Therefore we used the iodine method which is available for the determination of cystine in the presence of all the cleavage products of proteins. Folin-Loony's colorimetric method was also used for the sake of comparison. The two methods gave similar results in the proteins such as hair, wool, edestine, peptone and several kinds of muscle proteins, but in some proteins such as nuclein, casein, gelatine etc., which contain every small quantity of cystine, the colorimetric method gave much larger results than those of the iodine method. Especially was this the case with pure nucleic acid, guanine sulphate and glucose which, though they gave no csytine after acid hydrolysis by the iodine method, gave an appreciable amount of cystine by the colorimetric method. The authors came to the conclusion, therefore, that colorimetric method can not be used in some proteins, as there are some substances, other than cystine in the hydrolysed solution, which produce the color reaction like cystine.

3. The iodine method :- Although this has already been published by one of the authors (Okuda) in Japanese, for the sake of the reader's convenience we shall describe it here briefly. The basis of the method depends upon the fact that among the amino acid derived by the hydrolysis of proteins cysteine alone reacts very actively to the iodate-iodide-hydrochloric acid mixture in an acid solution. The principle

of the method is to titrate cysteine, freshly produced from cystine by reduction, with a standard iodate solution in the presence of some iodide and hydrochloric acid.

For the determination the following solutions are required :- (1), About 5% KI aqueous solution. (2), Exactly 4% HCl. (3), Exactly 2% HCl. (4), M/300 KIO_3, which is prepared by dissolving 2.14 g. of pure KIO_3 in 3 liters of exactly 2% HCl. This iodate solution should be standardized for cystine very carefully. Standardizing :- Dissolve 1.01 g. of cystine in 50 c.c. of about 5% HCl, add a few decigrams of zinc dust, leave it for 30 minutes at a room temperature (about 20° C), shake it now and then. Filter, wash and make it up to 100 c.c. with water. Take 1 c.c: of the freshly prepared filtrate immediately in a small dry Erlenmeyer flask or in a large test tube, mix with 19 c.c. of exactly 2% HCl, 5 c.c. of 5% KI and 5 c.c. of exactly 4% HCl, and then titrate with the iodate solution until a yellow color is produced. Then the required volume (4.65 c.c. at 17.5° C.) of the iodate solution corresponds to 0.0101 g. of cystine. The coefficient (c.c. of KIO_3) differs in different temperature, therefore it is conveninent to make a temperature curve in the beginning of experiments.

Procedure :- Take 1—10 g. of protein, boil it with about three times its weight of conc. HCl (sp. gr. 1.19) under a reverted condenser for about 20 hours. Decolorize it with the best charcoal by heating for 30 minutes, and then filter and wash with some boiling water. Add a little zinc dust to the filtrate for the reduction of cystine to cysteine, and leave it for 30 minutes at a room temperature. And then filter, wash and make it up to 100c.c. Take 1c.c. of the solution for the determination of the concentration of HCl in it, by means of the titration with a standard alkali solution. To the residual solution, add a calculated quantity of 20% KOH (or 20% HCl) to make it into a solution containing exactly 2% of free HCl. After ascertaining by titration that the solution contains exactly 2% HCl, take a definite volume (less then 20 c.c.) of the solution in a small dry Erlenmeyer flask, make it up to 20 c.c. with the exactly 2% HCl. Add 5 c.c. of 5% KI and 5 c.c. of 4% HCl, and then titrate with the standard KIO_3 solution, until the yellow color produced remains for one minute. If the temperature of the mixture was 17.5° C., and the iodate solution has been previously prepared so that its 4.65 c.c. corresponds to 0.0101 g. of cystine, the cystine content in the final 20 c.c. is as follows :-

$0.0101 \times$ required volume of $KIO_3/4.65$.

In this method the sample for a single determination should contain 2% HCl and 0.005—0.05 g. of cysteine in 20 c.c. of the solution. Especial attention is called to the fact that both the standardization of the iodate and the determination of the cystine must be made with an equal concentration of HCl (e.g, 2%), and with freshly reduced solutions. As cysteine is autooxidizable even in an acid solution, the reduced solution must be titrated within two hours.

If the original hydrolysed solution contains some cysteine, the results obtained as above express the sum of cystine and cysteine. But all the hydrolysed solutions investigated have contained no cysteine. For the purpose of testing cysteine in the hydrolysed solution, add a few c.c. of KI and a drop of the iodate. In the presence of cysteine the solution remains colorless, but in its absence it gives a yellow color. This reaction is more sensitive than the well known nitroprusside–reaction.

If cysteine is present in the hydroysed solution, the separate determination of cystine and cysteine is easily accomplished by the above method, titrating a sample –solution before and after the reduction, and calculating the difference of the two results. In this case 4.65 c.c. of the iodate solution corresponds to 0.01 g. of cysteine, which will be derived from 0.0101 g. of cystine by the reduction with zinc and HCl.

The Cystine Content of Muscle Proteins of Marine Animals.

By Zempei Ogura and Kō Fujikawa.

The cystine content of various muscle proteins of marine animals was determined by means of the iodine method described by Okuda, with the following results:–

Sample.	Cystine in 100 g. of moisture-free proteins.
Fish,	
Scomber japonicus	0.82
Spheroides sp.	0.87
Raja sp.	0.98
Squolus japonicus	0.95
Mollusc,	
Stichopus japonicus	0.75
Haliotis sp.	0.62
Arca inflata	0.59

Polypus octopodia	0.62
Cardium muticum	0.72
Crustacea,	
Neptunus pelagicus	1.12
Penaeus canaliculatus	1.19
Chionecetes phalangium	1.19

From the results of the analysis we see that the muscle proteins of crustacea contain much more cystine than those of the other marine animals. This fact seems to have some relation on the cause of the black-changing of the flesh of crab and lobster, which sometimes takes place in the cases of canning and cold storage.

Studies on the Enzyme Action.

I. On Phytase.

By Matsunosuke Kitagawa.
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The phytase solution and phytin were prepared by the following method :– For the preparation of phytase some rice bran was extracted with seven times its weight of 25% alcohol. To the extract one tenth its volume of saturated barium hydroxide and basic lead acetate solution were added, and the clear filtrate thus obtained was dialysed in a collodion membrane for two days. Such a phytase solution was more active than that prepared by the adsorptionmethod; it was pretty pure, containing no phytin and phosphate, and only minute traces of protein. The activity of phytase in water solution did not decrease for more than a week.

For the preparation of phytin, the crude phytin obtained from rice bran by the usual process was dissolved in 2% HCl, precipitated by barium hydroxide and washed several times with some hot water. The barium salt of phytin was again dissolved in 2% HCl, Ba removed by means of 6% H_2SO_4 quantitatively. To the clear filtrate some absolute alcohol was added and phytin was precipitated as a viscous mass, which was dried in a desiccator.

The phytin so prepared was very readily soluble in water, slightly acidic, and contains 49.5% P_2O_5.

For the estimation of phosphate, Brigg's modification of Bell and Doisys' colorimetric method (J. Biol. Chem., **53**, 13, 1920) was used.

At first the optimal pH for the enzyme action was determined in several buffer solution.

Phytase was less active in the citrate mixture than in the acetate or lactate mixture, but the optimal pH was the same in each case being 4.6—4.7. (Such a concentration of citrate as examined had no influence on the estimation of phosphate).

To a large extent, the concetration of phytase is exactly proportional to the amount of the phosphate produced, but in a very low concentration, the activity is not parallel with the concentration of the enzyme, but very much diminished.

There is a certain range of the optimal concentration of the substrate for the phytase reaction. Such an optimal range depends upon the concentration of enzyme, but not on the reaction stage and nature of the buffer mixtures which influence the action of the enzyme.

When the concentration of substrate is lower than the optimal, the time curve in the case of phytase reaction is logarithmic in nature as usually obtained, but in the optimal concentration the reaction velocity is independent of the concentration of the substrate and the curve becomes a straight line.

Studies on the Acids formed by Rhizopus species.

By Teizō Takahashi and Kinichirō Sakaguchi.

On the nature of the acid formed by the species of Rhizopus K. Saitō has mentioned already in 1904 in the description of his Rh. chinensis, (Cent. f. Bact. II. 1904, **13**, p. 54). Seven years later, Fe. Ehrlich found fumaric acid in the culture of Rhizopus nigricans in the medium containing glycocoll as its nitrogen source and this is the first instance ever since made on the formation of this acid from Rhizopus. He obtained oxyphenyl-lactic acid in the culture of the medium containing tyrosine instead of glycocoll, (Ber. 1911 **44**, Bd. III. S. 3737). In his second report, he mentions beside fumaric acid a trace of volatile acid, succinic acid, *l*-malic acid and *d*–lactic acid and moreover he adds that the some acids were affirmed to be produced by Rhizopus tritici Saitō. (Ber. 1919, Bd. **52**, S. 63).

On the other side, Goupil in 1911, isolated Succinic acid from the culture of Rhizopus Rouxii (Amylomyces Rouxii) (C. r. 1911, **153**, p. 1172—1174).

M. Hanzawa (Mycol. cent. 1912, Bd I, S. 76—92 et. 1914, Bd V. Heft. 5, S. 230, et. 1915 V. Heft. 6, S. 272—279). M. Yamazaki (Report of "Tōadōbun-shoin" in Japanese. 1911 et 1912), R. Nakazawa (Rep. of Gover. Resea. Inst. Formosa, Japan) and recently Y. Takeda (Rep. of Gover. Resea. Inst, Formosa, Japan. No. 5 of the Depert. of Indust. 1924). have studied on the formation of the acid by their Rhizopus but left aside the research of its nature.

The writers made researches on the formation and isolation of the acids formed from 17 species of Rhizopus and confirmed that we could devide these species into three types :– The first type forming fumaric acid mainly and none of lactic acid. The second type chiefly forming lactic acid and none of fumaric acid. Third one forms both fumaric and lactic acids.

The first type beside fumaric acid accompanies with a traces of citric, malic and tartaric acid (?). All species of the three types produce a trace of volatile acid and special species (Rhizopus chankuoensis) seems to form a trace of succinic acid beside fumaric acid.

To the first type twelve species of them such as Rhizopus Oryzae, R. tonkinensis vullemin, R. formosensis Nakazawa, R. formosensis var. chlamydosporus Yamazaki, R. candidus Yamazaki, R. chankunnensis Yamazaki, R. Hangchow Yamazaki, Rhizopus G. 34 Yamazaki, R. chiuniang Yamazaki, R. Delemar Wehmer et Hanzawa, R. niveus Yamazaki, R. albus Yamazaki are to be mentioned.

The second type involves :– Rhizopus salebrosus Yamazaki, Rhizopus G. 36. Yamazaki.

The third type implies three species :– R. chinensis Saito, R. liquefaciens Yamazaki, R. pseudochinensis Yamazaki.

The necessary and satisfactory conditions for the maximum formation of the fumaric acid is the ratio of nitrogen to carbon source in the nutrient. It is about 0.01 : 72 so in practice the most adequete quantity of the [former in the nutrient is adout one tenth of the common medium.

As the nitrogen source urea, zlycocoll, as Ehrlich has mentioned before, may be used as good one, but also gelatine, peptone, can play the some role as these and even inorganic nitrogen compound such as NH_4NO_3, $(NH_4)_2SO_4$ may be used as good as organic nitrogen under the rule mentioned above.

Glucose, cane sugar, and starch are very good carbon sources for the growth as for as well as the formation of fumaric acid.

The culture medium used was prepared as follows; water 1000 c.c., carbohydrate (glucose, cane sugar or starch) 100g., K_2HPO_4 0.15g, KH_2PO_4 0.15g, $MgSO_4$ 0.1g, $CaCl_2$ 0.1g, Fe_2Cl_6, NaCl trace, nitrogenous matter (peptone, urea, or gelatine) 1g, 1.2g, or 1g. and $CaCO_3$ 50g. (in some case this salt was omitted).

Isolation of Acids.

Fumaric acid: In the case when starch is used in the absence of $CaCO_3$ in the medium, for the isolation of the acid the concentration of the culture just by evaporating it is enough to get it in a free state, after the separation of mould growth from the culture on behalf of very tiny solubility of the acid. If $CaCO_3$ was added to the medium the acid becomes naturally into calcium salt, so calcium is removed from it by dilute sulphuric acid as sulphate. The filtrate from Ca-sulphate is acidified with sulphuric acid and the free acid is extracted by ether in Sudō-Kumakawa extraction apparatus. Raw acid, after recrystalisation from hot water or hot alcohol is purified. In the mother liquor citric, malic and succinic acid may be detected.

Lactic acid: This acid may be isnlated in the same way as in the case of fumaric acid.

If both fumaric and lactic acid occur in a mixed state they are separated as lead salts or barium salts; where just lead lactate is soluble in water or barium-lactate is saluble in 70—80 vol% of alcohol, when hot.

Identification.

Fumaric acid thus obtained sublimes at 200° C, melts at 285° C in a sealed tube. (After Fe. Ehrlich 280°).

The number of titration, substance taken 0.1g, 17.3 c.c. found, 17.24 c.c. calcul.

The hydrogen centents by elemental analysis;

Substance taken	0.2058 g
H_2O	0.0659 g
H found	3.55 %
H calculated	3.45 %

Silver salt. The silver was determined as chloride with the data of:-

	Substance taken.	Agcl.	Ag.	
$Ag_2C_4H_2O_4$ (dried at 110° C)	0.152 g	0.1314	found.	65.38 %
			calcul.	65.44 〃
$Ag_2C_4H_2O_4$ (dried at room t°).	0.0622 g	0.0515	found.	62.32 〃
$+H_2O$.			calcul.	62.04 〃

Dimethylester, $(CH_3)_2$. $C_4H_2O_4$ prepared from silver salt and methyliodide melts at 102° C. (Anschütz, 102° C. B. 12, 2282, 1879).

Dibrom–succinic acid, prepared by the addition of bromine to the substance melts at 255—256° C.

l–Lactic acid.

The acid from Rhizopus G. 36.

Zn–salt, water of crystallisation 12.18% (12.89% after Hoppe Seyler's Handbuch . S. 77. for active salt).

Zn–salt $[\alpha]^{15°} = +6.87$(4% salution).

The same from Rhizopus salebrosus.

Zn–salt. water of crystallisation 13.03%.

〃 $[\alpha]_D^{15°} = +6.25$(3.6% solution).

(After Hoppe–Seyler and Araki's result with *d*–lactated of 4.18% solution $[\alpha]_D = -7.55$ and of 9.08% solution $[\alpha]_D = -6.56$)

From the figures given above this acid must be a laevo–rotatory ones.

For citric, malic and tartric acid just a qualitative test was made, since the quantity was not enough. The cryitals obtained from the mother liquor of fumaric acid was taken, gave a precipitation of Hg–acetondicarbonate after Denige, which is a characteristic reaction of citric acid. (Abderhalden. Handbuch Arbeitm. Bd. II. S. 33). The same mass after neutralisation reduced palladium chlorid after the method of A. Hilger and H. Ley. (zeit. f. d. Unt. u. Nahr. u. Genus. Bd, 2. S, 795, 1899) This is characteritic to malate.

The same mass gave a violet red coloration when treated by resolcimol solution in sulphuric acid. This reaction is characteristic far tartric acid. (Roy. C. r. 1907, 145, 1285).

For the identification of these three acids a further researches is going on and its will be reported in future.

Sinomenine and Dehydrosinomenine.

Part III.

By Kakuji Gotō.

The method of kali fusion of sinomenine was improved, and the yield of the substance of m.p. 176° (formerly it was given as 169°, but the new m.p. was attained through the recrystallisation from acetone.) amounted to 30% of the theoretical.

The substance of m.p. 176° has the molecular formula $C_{16}H_{16}O_4$, contains two methoxyl groups and shows the reaction of a dipnenol, reminding particularly of the reaction of orthodiphenols such as apomorphine and pyrocatechine. The original carbonyl group contained in sinomenine seems to have been modified in this substance into a phenol group. I propose, therefore, to call this new substance sinomenol.

Sinomenol forms dibenzoyl (m.p. 207°) and diacetyl (m.p. 147°) derivatives. Methylation of sinomenol in an alkaline medium with dimethylsulphate leads to two substances (m.p. 115° and 240° respectively). Both the methylated sinomenols give no colour reaction of the original substance, have the same molecular formula $C_{18}H_{20}O_4$ and contain four methoxyl groups, estimated by the method of Zeisel. Dibenzoylated sinomenol melts at the same degree as the benzoylated substance obtained directly from sinomenine by the action of benzoylanhydride (see, this Journal 1924, vol. 1, p. 10 ; there the moleculer formula was errornously given as $C_{25}H_{20}O_5=5C_5H_4O$, but it is now ascertained from various analysis that it must have the formula $C_{30}H_{24}O_6=6C_5H_4O$. The admixture of these two substances did not change the melting point. There is, therefore, no dout about the fact that the latter substance is nothing but dibenzoylsinomenol. This fact was, moreover, confirmed by the debenzoylation of the latter substance, which gave sinomenol itself.

Diacetylsinomenol (m.p. 149°, the formula, $C_{20}H_{2}\,O_4$) is easily hydrolysed by alkali and then shows every colour reaction of sinomenol. It was found that the same diacetylsinomenol could be obtained directly from sinomenine by heating it with five parts of acetanhydride in a sealed tube for six hours at 175°.

Concordance of the results of elementary analysis of sinomenol, diacetyl, dibenzoyl and dimethyl sinomenols as well as dibenzoylsinomenolchinone leaves no doubt about the fact that sinomenol contains no vinyl side chain. The negative result obtained in an attempt to oxidise sinomenol in an acetone solution with permanganate also confirms this idea (ibid. p. 11).

Destillation of sinomenol with zinc dust gave crystals, which melted at 95°. It is almost certain that it is phenanthrene, since its picrate melts at 143° scharply.

From these facts sinomenol seems very likely to be a dioxydimethoxyphenanthrenedihydride.

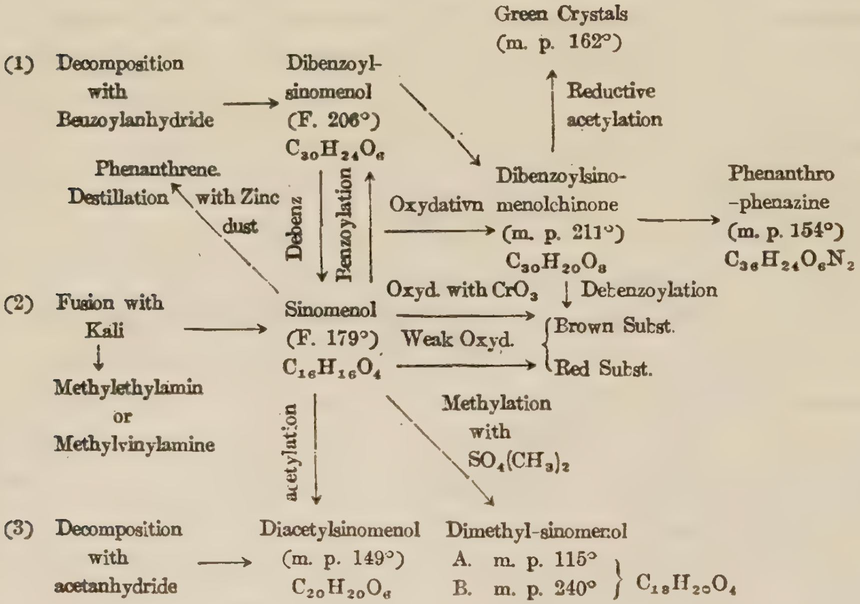
If sinonenol contains no vinyl side chain, then the chain of two carbon atoms must have been detatched from the phenanthrene nucleus together with the methylated nitrogen atom in the operation of the fusion with kali. It would then give rise to methylethanolamine or allied substance. The amine actually isolated was certainly not methylamine, and from the m.p. of the auri–and platinichloride, as well as the content of gold and platinum of these double salts, it is suspected to be methylethylamine or methylvinylamine.

Very striking is the ease with which sinomenol undergoes oxidation. Poured with 10% caustic soda and well stirred, it dissolves in it, taking at the same time a beautiful violet colour. The same colour is produced when sinomenol is oxidised with a very small quantity of permaganate, ammoniacal silver solution or alkaline ferricyanide. On the contrary, acidic oxidizing agents such as nitric acid, nitric acid and silver nitrate and gold chloride give a deep red colour to the acetone solution of sinomenol. These changes seem to be one and the same. For, from the violet aqueous solution a red precipitate is thrown down on addition of an acid and is extracted then by organic solvents such as ether. From the alkaline violet aqueous solution neither ether nor chloroform can extract the substance, so it seems that in the violet solution there is the formation of salt of the oxidised substance.

As dibenzoyl and dimethyl sinomenols give no such reaction, the two hydroxyl groups of the sinomenol must be in a direct relation with the formation of this coloured substance. The deep colour reminds us of the existence of a chinone. But the mild reagents such as air oxygen or ammoniacal silver solution would not be able to attack the carbon atoms in the positions 9 and 10 in phenanthrene nucleus. It seems, therefore, very likly that the two hydroxyl groups in sinomenol lie in an orthoposition each other. Moreover, there is probability that a new hydroxyl is introduced (as is the case with 1—8 dioxynaphtaline), otherwise it would not be able to form a violet salt. This was indirectly proved by the fact that the reductive acetylation of this red substance leads to an acetyl compound entirely different (m.p. over 180°, as yet amorphous) from the diacetylsinomenol.

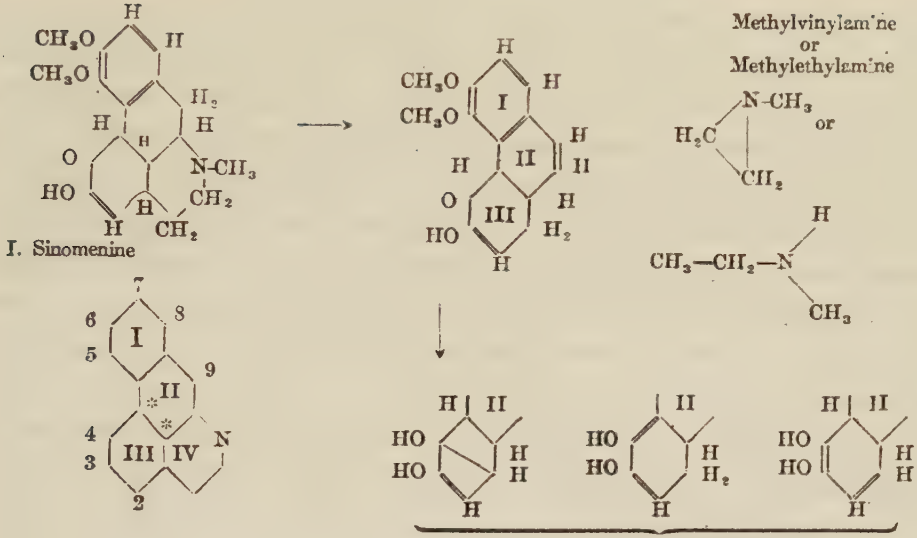
The oxidation of sinomenol with chromic acid in glacial acetic acid gives a small quantity of the above red substance and much of a brown sunstance, which is very insoluble in this solvent. This substance seems to be very likely the same substance with that obtained by the alkaline hydrolysis of dibenzoylsinomenolchinone. Neither the red substance nor its derivatives have not yet been obtained in crystals.

The relation of the above given substance each other can be seen clearly from the following table.



On the constitution of Sinomenine.

It is now clearly established that sinomenine ($C_{19}H_{23}O_4N$; with two methoxyls, one carbonyl and N-methyl) is a tetrahydroisoquinoline alkaloid belonging to a phenanthrene group. Its constitutional formula may be given provisionally as follows.

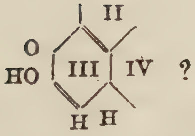


I. Sinomenine

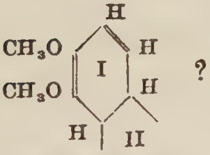
Methylvinylamine or Methylethylamine

$CH_3-CH_2-N(H)-CH_3$

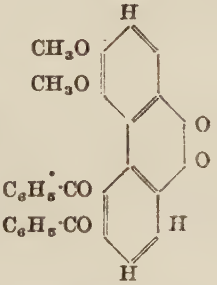
II. Sinomenol



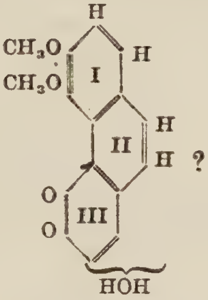
Dehydrosinomenine



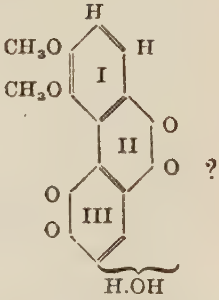
Hydrosinomenine



Dibenzoylsinomenol-chinone



Red Substance.



Brown Substance.

The position of the two methoxyls and the benzenoid character of the nucleus, to which these two methoxyls are attached, have been selected hypothetically according to the constitution of the most of the opium alkaloids.

The position of the carbonyl (4) and of the hydroxyl (3) was selected according to the behaviour of the sinomenol and the red substance derived from it. For, if sinomenine has the foumula I, then sinomenol must have the formula II.

This explains well the formation of dibenzoyl, diacetyl and dimethyl derivatives of sinomenol. That the methylaminoethyl group $(-CH_3N-CH_2-CH_2-)$ is attached to this skeleton in positions 10 and 1 (and particularly N in 10) is deduced from the following facts. Benzoylsinomenine contains only one benzoyl group (J. of chem. Soc. of Japan, 1924, June number), and sinomenine when methylated, takes only one methyl group and this monomethylsinomenine still forms an oxime (this point is not yet proved by the auther. But compare. Kondo and Ochiai, J. of pharm. Soc. of Japan, 1923, No. 503. p. 20) These facts show that the paraposition of the carbonyl group in sinomenine is occupied, so as it gives hindrance to the desmotropic change of the carbonyl group, observed in sinomenol. (The alternative that N is attached at the position 1 and C in 10 is very unlikely from the known facts about the phenenthrene alkaloids. But the decisive conclusion can be given only when sinomenol carboxylic acid is obtained.)

The formation of the red substance from the sinomenol makes us believe that the original carbonyl and the hydroxyl must lie in an orthoposition each other. And the fact that sinomenine gives an intense diazoreaction requires that the orthoposition of the hydroxyl must be free. Hence the hydroxyl in position 4. The unsaturation between 2 and 3 is assumed from the phenolic character of the same hydroxyl.

Although the above formula holds good in main respects, it has naturally its difficult sides also. For, the formation of dehydrosinomenine ($C_{19}H_{21}O_4N$) is well explained, if we assume by the dehydrogenation the hydrogen atoms marked with asterisk are taken away. But how it can account for the formation of hydrosinomenine ($C_{19}H_{25}NO_4$) ? This substance gives also an intense diazoreaction as well as ferric chloride reaction, so that the unsaturation between 2 and 3 must remain untouched. Will the benzenoid nucleus be reduced partially by moleoular hydrogen and colloidal palladium ? This is somewhat singular, but no other explanation can be put forward easily.

The relative position of bromine atom in two different isomeric monobromsinomenines (ibid. p. 8.) is not yet more than imagination. The isomer of lower m. p. (153°), which gives ferric chloride and diazoreaction, may contain the bromine atom in the nucleus I, and the higher m. p. (214°) may contain it in the position 2 of the nucleus III. Yet it does not explain why the ferric chloride reaction disappears also in this isomer.

The decision whether the above formula is to be regarded correct or not may be given in a degree by the oxydative decomposition of the sinomenol or its derivatives. This is my next task and will be, I hope, reported shortly after.

EXPERIMENTAL.

Sinomenol, by kali fusion of sinomenine. 10 grs. of sinomenine, 100 grs of potassium hydroxide and 50 grs of water were heated in a distilling flask on a glycerine bath at 180-200°. The liquid distilling over was collected in a test-tube, and the gas was caught in two per cent hydrochloric acid. After two hours heating, the content of the flask was made acidic with 15% sulphuric acid and extracted twenty times with ether. When the ether was dried and distilled off, sinomenol crystallised out in the red substance, which could be washed out with methyl alcohol. It can be recrystallised from glacial acetic acid, ethyl alcohol or acetone, but the oxidation sets in sometimes during the operation. M.p. 176°. Yield 2.4 grs., i.e. 30% of the theoretical. It has the formula $C_{16}H_{16}O_4$ ($C=70.39\%$; $H=6.3\%$; $C_{16}H_{16}O_4$ requires $C=70.58\%$; $H=5.89\%$.) and contains two methoxyl groups (20.73% ; calculated, 22.79%,) For the colour reactions of sinomenol, see the theoretical part.

Sinomenol, by debenzoylation of the dibenbzoylsinomenol obtained directly from sinomenine by benzoylanhydride. 0.6 gr. of the dibenzoylsinomenol was boiled with 9 grs of 66% caustic kali about fifteen minutes. The solution was made acidic amd extracted five times with ether. The residue, when the ether was evaporated, was washed with a small quantity of methyl alcohol to free it from benzoic acid. Yield 0.1 gr. M. p. 176°. (unaltered by the admixture of sinimenol obtained by the kali fusion).

The methyl alcoholic filtrate is coloured intensely red. This shows that the red substance can also be formed from sinomenol or rather, that the red substance formed in the kali fusion of sinomenine is perhaps the same with that obtained by the oxidation of sinomenol, so that the red substance is formed secondarily from sinomenol in the kali fusion of sinomenine.

Diacetylsinomenol, by the acetylation of sinmenol. 1.5 grs of sinomenol, 3 grs. of anhydrous sodium acetate and 20 grs. of acetanhydride were boiled for six hours with air condensor. Poured into water, diacetylsinomenol separated out in crystalline powder. Recrystallised from fifteen parts of methyl alcobol, it crystallises in beautiful prisms. M. p. 149°. It has the molecular formula $C_{20}H_{20}O_6$ ($C=67.55\%$; $H=5.93\%$; $C_{20}H_{20}O_6$ requires $C=67.41\%$; $H=5.62\%$) and contains two acetyl groups(22.76%, as the result of hydrolysis by cold sodium ethylate ; calc. as two acetyl groups 24.15%). It is hydrolysed by 10% caustic soda and then shows the colour reaction of sinomenol.

Diacetylsinomenol, by the action of acetanhydride on sinomenine. 2 grs of sinomenine mixed with 10 c.c. acetanhydride were heated in a sealed tube at 175° during six hours. The brown solution was poured in water and well stirred. Crude yellow powder thus ontained amounted to 2 grs. Twice recrystallised from glacial acetic acid the yield was 0.4 grs, thus ca. 20%. of the theoretical. M.p. 149°. Admixture with the diacetylsinomenol obtained by the acetylation of sinomenol did not alter the m.p. The scanty yield is perhaps due to the difficulty of the isolation.

Dibebenzoylsinomenol by debenzoylation of sinomenol. Sinomenol seems not much to be benzoylated by heating it with benzoylanhydride in boiling water-bath. Better it is benzoylated in ethylbenzoate with benzoylchloride and caustc alkali after the manner of Schotten-Baumann. Recrystallised from glacial acetie acid, it melts at 207°. Yield ca. 70% of the theoretical. Mixed with the dibenzoylsinomenol obtained directly from sinomenine by benzoylanhydride, its m. p. does not change. Other proprties as well as the analytical results are identical in the both samples.

Analysis. C=75.48% ; H 5.27% ; $C_{30}H_{24}O_6$ requires C=75.00% ; H=5.00%.

The hydrolysis of the dibenzoylsinomenol is not easy. Weak alkali does not attack it, while the strong destroys the molecule of sinomenol and give rise to volatile acids. But from the results of the above analysis and the behaviour of the other derivatives of sinomenol, it is certain that it contains two benzoyl groups.

Dibenzoylsinomenolbhinone from the sinomenol obtained by kali fusion. Sinomenol was benzoylated as above and oxidised to chinone. The intense reddish yellow colour revealed at once that it is the same substance with that produced from dibenzoylsinomenol obtained directly from sinomenine by the action of benzoylanhodride. M. p. 211°. The mixture of the both samples melted at the same degree. (Note. The formula of this chinone now must be $C_{30}H_{20}O_8$)

Reduction of dibenzoylsinomenolchinone. When dibenzoylsinomenolchinone is boiled with glacial acetic acid and zinc dust, its reddish yellow colour disappears quickly, showing that reduction is going on. But on filtering, it takes a deep green colour and then recovers instantly its original reddish yellow colour. The crystalls, which were formed on cooling, were reddish yellow and melted at 211°.

The deep green colour is perhaps given by the formation of chinhydrone like substance in the way of autoxidation of the reduced chinone. The latter was fixed by the reductive acetylation.

Reductive acetylation of dibenzoylsinomenolchinone. 0.2 gr. of this substance was reductively acetylated by boiling it with 5 grs of acetanhydride and 0.5 gr of zinc dust for six hours. The precipitate, formed on addition of water. was recrystallised from methyl alcohol. It forms fine prisms, collected in rosettes. M. p. 164°. Analysis is not yet performed on account of the scarcity of the substance.

Methylation of sinomenol. 2 grs of sinomenol were methylated with 30 grs of 10% caustic soda and 10 grs of dimethylsulphate, added in four portions. After standing overnight, it was diluted with water, filtered and the residue was washed with water, until the filtrate showed no longer alkaline reaction. The residue was extracted with boiling methyl alcohol. On evaporating methyl alcohol, slightly coloured crystals were obtained. M. p. 115° (not sharp). It has the molecular formula $C_{18}H_{20}O_4$ (C=72.59% ; H=6.51% ; $C_{18}H_{20}O_4$ requires C 72.00% ; H=6.71%). and contains four methoxysl(40.36% ; calc. 41.33%).

The residue, which was insoluble in boiling methyl alcohol, was extracted with boiling glacial acetic acid, from which beautiful needles crystallised out. M. p. 240°. It has the same molecular formula as the above. (C=72.01% ; H=6.39%) and contains four methoxyls also (40.71% ; calc. 41.33%).

The both substances dissolve in concentrated sulphuric acid with brown colour and it turns green when slightly warmed. But on addition of water a striking difference is observed. The substance of m. p. 115° decolourises at once, while the substance of m. p. 240° takes a beautiful purple colour, giving a reddish amorphous precipetates after a time..

The reason, why these two methylated substances are prodnced, is not yet accounted for.

Distillation of sinomenol with zinc dust. 1 gr of sinomenol was distilled with much zinc dust in a tube, in an atmosphere of hydrogen. 0.1 gr of crude crystals was obtained, which, recrystalliied from alcohol melted at 95°. Its picrate melted sharply at 143°, so it is almost certain that we have phenanthrene here.

Nitrogenous decomposition products. In the kali fusion of sinomenine, amount of the escaping gas, which was absorbed in dilute hydrochloric acid was very small. On evaporating the hydrochloric acid solution, only a thin crust was observed on the bottom of the watch-glass. This part was, therefore, thrown away.

The liquid condensed in the test tube was filtered and extracted five times with ether. The ethereal extract was shaken with dilute hydrochloric acid and the latter was evaporated.

The aqueous part was also neutralised with hydrochloric acid and eveporated. From these two portions the platinichlorides were prepared separately and their m. p. was compared. As both these platinum double salts melted and decomposed sharply at 224°, it was clear that we had the same amine in the ethereal extract as well as in the aqueous solution. The gold double salt melted at 179-180° without decomposition. The content of Pt and Au was respectively 37.28% and 49.35%.

From the centent of Pt and Au in these double salts. it may be inferred that we have here methylethylamine or methylvinylamine. The m. p. of the gold double salt coincides with that of methylethylamine aurichloride, but the m. p. of the platinichloride shows the difference of twenty degrees from that of the methylethylamine platinichloride. As regards the suspected methylvinylamine it seems that this amine has not yet been described in the literature.

The hydrochloride of this amine is very hygroscopic and deliquates in a few minutes in the air and its m. p. can not be dertermined easily.

The theotetical yield of this amine from 55 grs of sinomenine, based on the yield of sinomenol is 2.97 grams. Calculated from the amount of hydrochloric acid required to neutralise the alkaline destillate, the actual yield seems to be not more than 2.5 grs. So it may be concluded that almost all the amine produced when sinomenine decomposed into sinomenol was collected in this destillate. When the sulphuric acid solution, from which sinominol was isolated, was evaporated down to a small volume, made alkaline and distilled, it gave only a small quantity of basic substance.

The red substance. The red substance is formed as stated above when sinomenol is exposed to the action of milder oxydising agents. It is also formed at the time of kali fusion of sinomenine. It is not yet obtained in crystalline form.

The red substance is very sensitive to the nitric acid. Warmed with a small quantity of the latter, it turns yellow and a faint aromatic odour is set out.

The red substance dissolves green in concentrated sulphuric acid, but on addition of water a red amorphous substance is again precipitated.

The red substance is best prepared by the action of ammoniacal silver solution on sinomenol. 1.5 grs of sinomenol are dissolved in 100 c.c. of alcohol, added with alcoholic solution of 3 grs of silver nitrate and then made alkaline with concentrated ammonia. After several hours the violet solution is made acidic and filtered. The silver is washed well with alcohol. The combined alcohol is poured into ten volumes of water. The red amorphous precipitate thus obtained is collected and washed. Yield ca. 0.6 gr, i.e. 30%.

Acetylation of red substance. The red substance was acetylated as with sinomenol. Yellow crystalline powder was obtained, which melted at about 80°. It is easily hydrolysed by alkali and then on acidifying, it recovers its original red colour.

Reduction of the red substance. The red substance is decolourised by glacial acetic acid and zinc dust. But on standing it recovers its colour after some time so that the reduced substance can not be isolated in a usual way.

Reductive acetylation of the red substance was carried out in a way exactly similar to that of dibenzoylsinomenolchinone. Yellow crystalline powder was obtained which was quite differet from diacetylsinomenol and melted at about 180°. This shows perhaps that a new hydroxyl was introduced in the red substance while the original hydroxyls were oxidised to a chinone group.

The brown substance from sinomenol by the oxidation with chromic acid. 0.4 gr impure sinomenol was oxidised in boiling glacial acetic acid with 0.2 gr chromic acid. On cooling 0.1 gr of the brown substance separated out. This substance is very difficultly soluble in methyl alcohol also. Judged from the condition of its fromation the brown substance is perhaps identical with the debenzoylated dibenzoylsinomenolchinone.

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